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TECHNICAL MANUSCRIPT 541

**CROSS IMMUNITY AMONG
STRAINS OF CHLAMYDIA PSITTACI**

**John R. Mitzel
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JUNE 1969

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DEPARTMENT OF THE ARMY
Fort Detrick
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TECHNICAL MANUSCRIPT 541

CROSS IMMUNITY AMONG STRAINS OF CHLAMYDIA PSITTACI

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Medical Investigation Division
MEDICAL SCIENCES LABORATORIES

Project 1B662706A072

June 1969

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

The degree of cross protection induced by immunization with vaccines derived from a representative group of psittacosis, human pneumonitis, and related chlamydiae is reported. Nonviable vaccines were prepared by growing the organisms in human diploid cell culture WI-38, followed by inactivation with formaldehyde. Swiss-Webster mice were immunized by three intraperitoneal injections of vaccine and challenged intraperitoneally after 3 weeks with graded doses of the infectious chlamydiae. Results were expressed as the protective index, which represents the difference between the log lethal titers of the challenge suspension in normal and in immunized mice. Most vaccines were effective against homologous challenge, with protective indices as high as 6.4. Some degree of cross protection was observed in many of the combinations tested. The degree of relationship ranged from close, as in the case of Borg and the NJ strain of turkey ornithosis, to remote, as in the case of Borg and WC, a bovine isolate. The Havlik strain was poorly protected against with any vaccine, including the homologous. Polyvalent vaccines may be necessary if protection against a broad spectrum of strains is required.

CROSS IMMUNITY AMONG STRAINS OF CHLAMYDIA PSITTACI*

Previous reports from our laboratories have described growth of Chlamydia in diploid human cell strain WI-38.^{1,2} Formalin-inactivated vaccines derived from strains propagated under these conditions protected mice against appreciable levels of intraperitoneal challenge and against lower but significant levels of respiratory challenge.

The various vaccines warrant consideration for practical immunization. Accordingly, attention has been given to the degree of cross protection against other strains of Chlamydia demonstrable with univalent preparations. Members of the genus Chlamydia share a common heat-stable antigen and also possess strain-specific antigens associated with the cell wall.³⁻⁵ The importance of these antigens in protection against infection has not been established, and at present the degree of cross protection cannot be inferred from serological studies.

Previous exploratory experiments had indicated that vaccines derived from the Borg and 6BC strains gave only partial cross protection. Accordingly, a more extensive study was made of the cross protection among a group of representative strains of Chlamydia.

Strains of various origin were obtained from Dr. L.A. Page, Dr. Paul Arnstein, and from the Fort Detrick collection. Strains were maintained as 50% chicken yolk-sac suspensions and stored at -70 C. A summary of strains used and their origin and pathogenicity for embryonated chicken eggs, mice, and guinea pigs is shown in Table 1.

The study included three strains isolated from human pneumonitis: Borg, San Francisco, and meningopneumonitis. Of these, Borg was highly pathogenic for embryonated eggs, mice, and guinea pigs. San Francisco and meningopneumonitis killed mice at low dilutions, and 50% yolk-sac suspensions of San Francisco also killed guinea pigs. The three strains of turkey ornithosis, New Jersey, Virginia, and Havlik (isolated in Oregon), were all pathogenic for eggs, mice, and guinea pigs. Of the remaining strains of various origins, 6BC, California pigeon, and the bovine isolate WC were pathogenic for eggs and mice but not for guinea pigs. Feline pneumonitis was pathogenic for chicken eggs but not for mice or guinea pigs. The strains also were tested in accordance with the criteria for differentiation of species in the genus Chlamydia as proposed by Page.^{6,7} On the basis of these tests, all strains were placed in the species Chlamydia psittaci, which are insensitive to sodium sulfadiazine and do not produce glycogen.

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TABLE 1. CHARACTERISTICS OF PSITTACOSIS STRAINS

Strain	Origin	YSLD ₅₀ /ml	MIPLD ₅₀ /ml	Lethality for Guinea Pigs
Borg	Human pneumonitis	9.1	8.6	+
San Francisco	Human pneumonitis	7.6	1.1	±
MnPn	Man (or ferret?)	9.4	1.8	-
NJ-1	Turkey ornithosis (New Jersey)	8.5	8.8	+
VT-1	Turkey ornithosis (Virginia)	8.7	8.5	±
Havlik	Turkey ornithosis (Oregon)	8.4	8.8	+
6BC	Parakeet	9.5	7.4	-
CP-3	Pigeon ornithosis (California)	7.6	2.1	-
WC	Bovine	8.5	8.3	-
FP	Respiratory infection of cats	8.9	<1.0	-

Each strain was also tested in various cell lines to determine the host range. Growth in the tissue cultures was determined by the presence of CPE. Results are summarized in Table 2.

CPE appeared between 24 hours and 12 days in all cases except meningo-pneumonitis in LLC-MK₂. In general, the strains that were highly pathogenic for mice and guinea pigs caused CPE more rapidly than the strains with low pathogenicity.

Nonviable vaccines were prepared in WI-38 human diploid cell cultures. The cell sheets were infected with 1:100 dilutions of stock yolk-sac suspensions and incubated with BME maintenance medium until the cell sheets showed 2+ to 3+ CPE. The culture supernatant liquid was removed and inactivated with 0.02% formaldehyde. Nonviability was established by the absence of lethality for embryonated eggs.

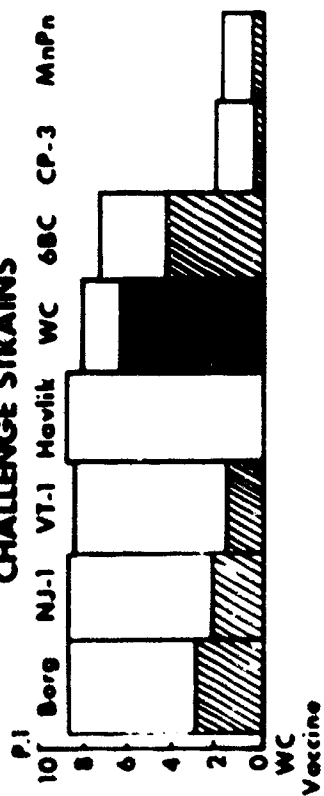
TABLE 2. APPEARANCE OF CPE WITH PSITTACOSIS STRAINS
IN VARIOUS CELL CULTURES

Strain	Infective Dose, \log_{10} YSLD ₅₀ /ml	Days to 2+ CPE					
		WI-38	L Cells	BHK ₂₁	VERO	GMK	LLC-MK ₂
Borg	7.1	3	3	2	2	3	4
San Francisco	5.6	6	3	3	4	4	4
MnPn	7.4	12	8	7	7	5	No CPE
NJ-1	6.5	6	3	2	4	3	4
VT-1	6.7	6	2	2	3	2	2
Havlik	6.4	4	1	2	2	2	2
6BC	7.5	5	4	3	3	4	6
CP-3	5.6	10	9	7	7	6	8
WC	6.5	7	5	4	4	4	7
FP	6.9	3	4	7	7	3	4

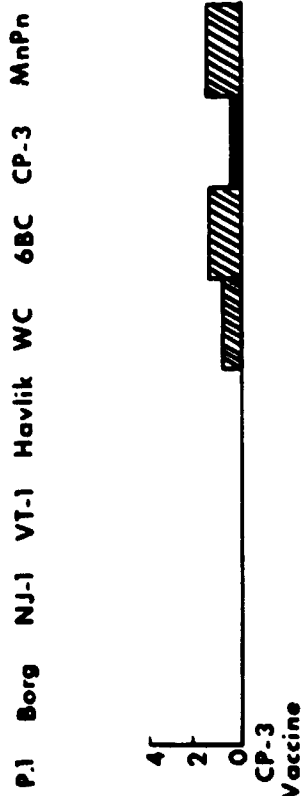
Groups of Bagg strain Swiss-Webster mice from the Fort Detrick colony were immunized with three intraperitoneal injections spaced a week apart. Three weeks after the last injection, the mice were divided into groups and challenged intraperitoneally with graded doses of infectious suspensions of the various strains. Deaths were recorded for 14 days. Results were expressed as the protective index, which represents the difference between the \log_{10} LD₅₀/ml of the challenge suspension in unimmunized and in immunized mice. The results of the homologous and heterologous challenge are presented in Figure 1.

Both similarities and differences among strains are evident. The strains appeared to fall into two groups. The first group contained the Borg, NJ ornithosis, VT ornithosis, and Havlik strains. These vaccines all gave high levels of protection to other members of the group, except that Havlik was difficult to protect against with any vaccine. These strains gave lower levels of protection to members of group 2. The results are shown in Figure 1. Group 2 contained the bovine isolate WC, 6BC, SP, and California pigeon strains. This group gave its highest protective levels to members of this second group and irregular low level protection to group 1. The MnPn and FP vaccines showed little protection of any kind.

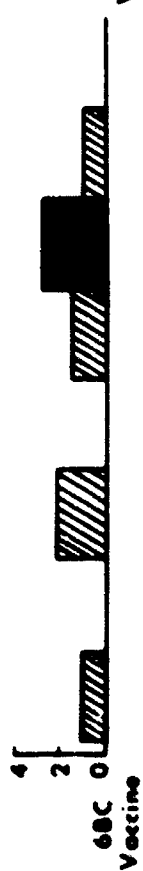
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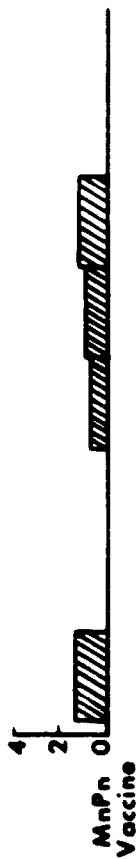
CHALLENGE STRAINS



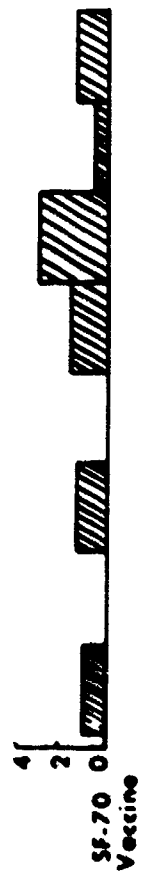
P.1 Borg NJ-1 VT-1 Havlik WC 6BC CP-3 MnPn



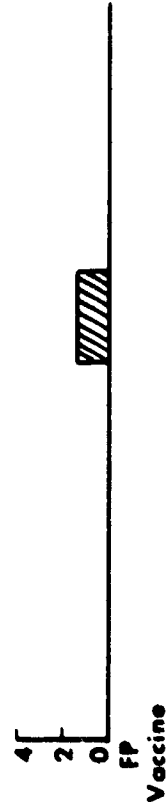
P.1 Borg NJ-1 VT-1 Havlik WC 6BC CP-3 MnPn



P.1 Borg NJ-1 VT-1 Havlik WC 6BC CP-3 MnPn



P.1 Borg NJ-1 VT-1 Havlik WC 6BC CP-3 MnPn



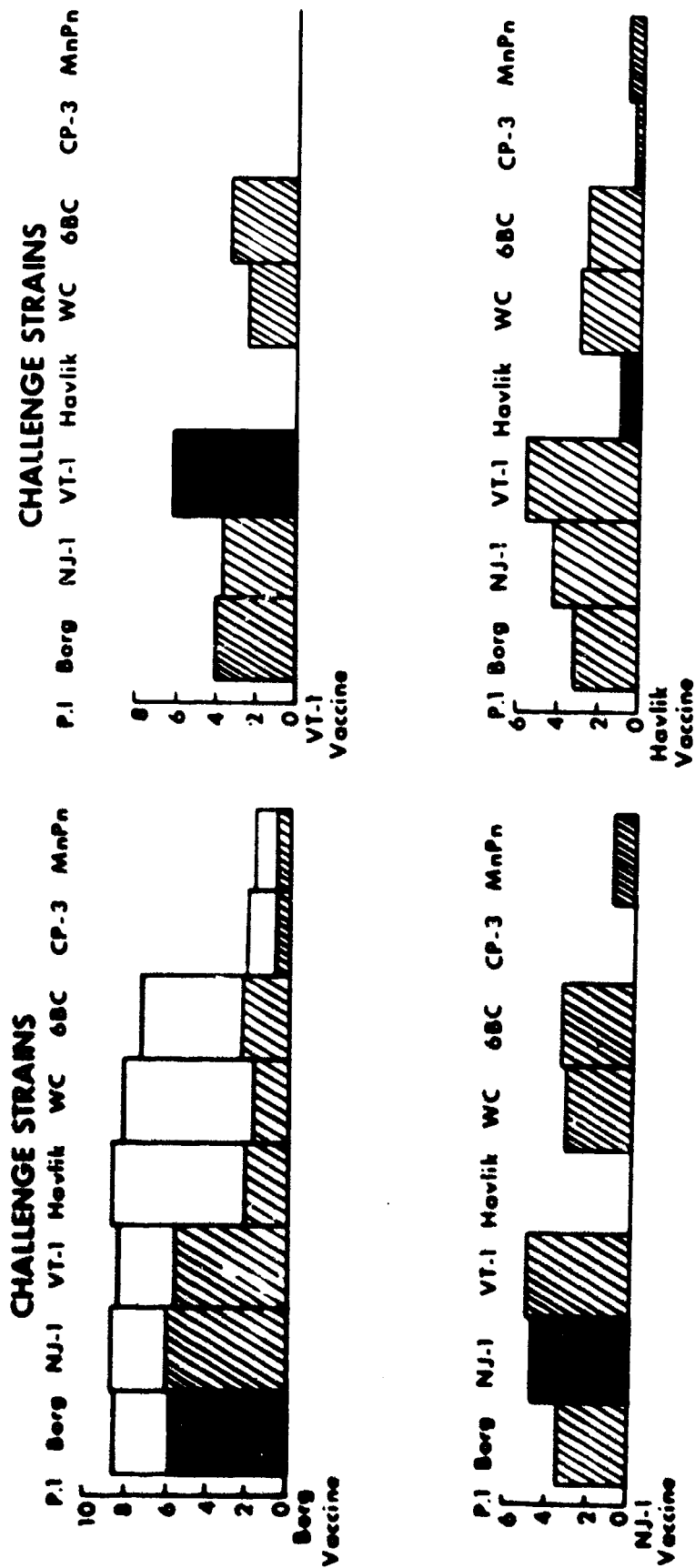


FIGURE 1. Protection Afforded Mice by Various Chlamydial Vaccines.

Some degree of cross protection was observed among almost all strains tested. The degree of protection ranged from the high degree given by the Borg and New Jersey strains to a very low level of protection given by meningopneumonitis and feline pneumonitis. The Borg strain seems to be closely related to the turkey ornithosis strains, but WC, 6BC, SF, and CP are separated from these strains by a low level of cross protection.

It is apparent that no one strain of the psittacosis group will give a broad spectrum of protection and that at least a bivalent vaccine will be necessary if protection against this group is desired.

In summary, cross immunity studies were conducted with a series of psittacosis strains of various origins. Mice were immunized intraperitoneally with nonviable vaccines and tested for their ability to withstand homologous and heterologous challenge. Most strains of psittacosis provided some degree of cross protection, and the strains of avian origin appeared to have a broader antigenic pattern than those of other origins. To give significant protection against a broad spectrum of psittacosis strains, a polyvalent vaccine may be required.

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